Supporting Information

Using Boronolectin in MALDI-MS Imaging for the Histological Analysis of Cancer Tissue Expressing the Sialyl Lewis X Antigen

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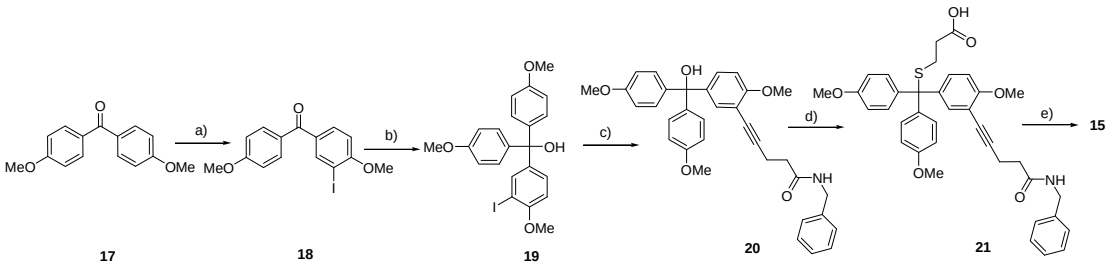
General Information

Solvents and reagents were purchased from VWR, Acros, or Aldrich and used without purification unless specified otherwise. When necessary, solid reagents were dried under high vacuum. Reactions with compounds sensitive to air or moisture were performed under argon. Solvent mixtures are indicated as volume/volume ratios. Thin layer chromatography (TLC) was run on Sorbtech W/UV254 plates (0.25 mm thick), and visualized under UV-light or by a Ce-Mo staining solution (phosphomolybdate, 25 g; $Ce(SO_4)_2.4H_2O$, 10 g; H_2SO4, 60 mL, conc.; H_2O, 940 mL) with heating. Flash chromatography was performed using Fluka silica gel 60 (mesh size 0.040-0.063 mm) using a weight ratio of ca. 30:1 for silica gel over crude compound. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer in deuterated chloroform ($CDCl_3$), methanol-d_4 (CD_3OD), or DMSO-d_6 with either tetramethylsilane (TMS) (0.00 ppm) or the NMR solvent as the internal reference.

Experimental Section



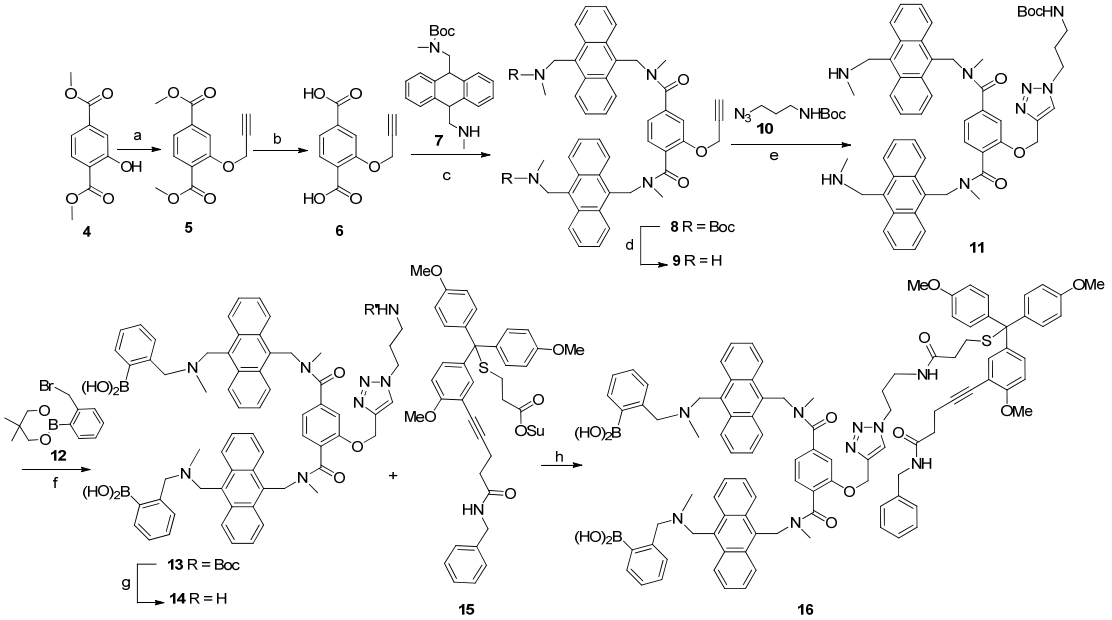
a) I_2, HNO_3, dioxane, H_2O, 60 °C, 40%; b) 4-methoxyphenylmagnesium bromide, THF, rt,90%; c) *N*-benzyl pentynoic amide, DMF, CuI, Et_3N, $Pd(PPh_3)_4$, rt, 67%; d) 3-mercaptopropionic acid, TFA, CH_2Cl_2, rt, 2h, 62%;e) EDCI, SuOH, CH_2Cl_2, rt, 3h, 72%.

Scheme S1. Synthesis of DMTr-S-ester (**15**) for mass tag conjugate

1. Synthesis of DMTr-S-ester (15) for mass tag conjugation

As shown in Scheme S1, iodination of diarylketone compound **17** afforded monoiodo compound **18**. Subsequent Grignard addition gave trityl compound **19**. A side arm was installed through Sonogashira reaction to give **20**, which was reacted with 3-mercaptopropionic acid in the presence of trifluoroacetic acid (TFA) to give DMTr-S-acid (**21**). The carboxyl group was then activated through conversion to its *N*-hydroxysuccinimide ester (DMTr-S-ester, **15**).

2. Synthesis of boronolectin-DMTr mass tag conjugate



a). propargyl bromide **7**, K_2CO_3,CH_3CN, reflux 24 h, quantitative; b). 2N NaOH, CH_3OH, reflux 3h, 84% c) DCM, EDCI, HOBT, DMAP, 88%; d) TFA, DCM, 82%; e) CuI, DIPEA, DMSO, 80 °C, microwave, 97%; f). K_2CO_3, CH_3CN, 79%, g) TFA, DCM, 73%, h). Et_3N, DMF, DCM, 36%

Scheme S2 Synthesis of boronolectin-DMTr mass tag conjugate

By reacting **4** with propargyl bromide, the alkyne side chain was introduced to give **5** in quantitative yield. The subsequent hydrolysis of **5** under basic conditions gave diacid linker **6** with an alkyne handle in 84% yield. Compound **7** was coupled with diacid linker **6** using 1-(2-dimethylamino-propyl)-3-ethyl carbodiimide hydrochloride (EDCI) as the activating reagent to furnish compound **8** with a di-anthracene group and an alkyne handle in 88% yield. Deprotection of **8** with TFA in dichloromethane (DCM) gave **9,** which was reacted with azido compound **10** under microwave conditions to give triazole compound **11**. The boronic acid moiety was attached through alkylation using potassium carbonate as the base to give the bisboronic acid compound **13.** Deprotection of the Boc group with TFA in DCM gave free amine **14**, which was then conjugated with DMTr-S-ester **15** to give the final mass spectrometric tag conjugate **16**.

Synthesis

3-Iodo-4,4'-dimethoxybenzophenone (18)

To a solution of 4,4'-dimethoxybenzophenone (**17**) (1.8 g, 7.5 mmol) in dioxane (10 mL), iodine (1.0 g, 3.9 mmol) was added at 60 ^{o}C. After the mixture was stirred for 15 min, water (2 mL) was added followed by the addition of 58% HNO_3 (4.1 mL) in a drop wise fashion within 1 h. The reaction was keep stirring at 60 ^{o}C for about 6 h until iodine color disappeared. Nitrogen was bubbled through the reaction mixture until the cessation of brown gas emission. Then the mixture was diluted with water (100 mL) to afford pale yellow precipitates, which were collected and washed with 5% $NaHCO_3$ aqueous solution and water. Re-crystallization from EtOH (20 mL), followed by column chromatography purification with hexane/EtOAc (10:1) gave white needles (1.1 g, 40% yield). ^{1}H NMR (DMSO-d_6): 8.11 (s, 1H), 7.73 (m, 3H), 7.12 (m, 3H), 3.95 (s, 3H), and 3.87 (s, 3H) ppm. MS-ESI: $[M+H]^+$ (m/z, 369.0).

3-Iodo-4-methoxyphenyl-bis(4-methoxyphenyl)methanol (19)

To a solution of 3-iodo-4,4'-dimethoxybenzophenone (**18**) (1.0 g, 2.7 mmol) in dry THF (20 mL), 1M solution of 4-methoxyphenylmagnesium bromide in THF (5.5

ml, 5.5 mmol) was added in one portion at 0 ^{o}C. After stirring at RT overnight, the reaction solution was diluted with 150 mL of saturated $NaHCO_3$ solution and extracted with EtOAc (50 mL × 3). The EtOAc layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography with hexane/EtOAc (10:1) to give white crystals (1.2 g, 90% yield). ^{1}H NMR ($CDCl_3$): 7.77 (s, 1H), 7.16 (m, 5H), 6.87 (m, 4H), 6.74 (d, J = 5.6 Hz 1H), 3.89 (s, 3H,), and 3.82 (s, 6H) ppm. MS-ESI: $[M+H]^+$ (m/z, 477.3).

_N_-Benzyl-3-{2-methoxy-5-[hydroxyl-bis(4-methoxyphenyl)methyl]-phenyl}-4-pentynoic amide (20)

To the solution of compound **19** (530 mg, 1.11 mmol) and *N*-benzyl pentynoic amide (219 mg, 1.17 mmol) in DMF (6 mL), $Pd(PPh_3)_4$ (116 mg, 0.1 mmol), CuI (38 mg, 0.2 mmol), and Et_3N (235 uL, 1.68 mmol) were added in sequence under N_2 protection. The reaction mixture was stirred at room temperature overnight, then diluted with water (100 mL) and extracted with EtOAc (60 mL × 3). The EtOAc extract was washed with water (50 mL), 0.1 M solution of $(NH_4)_2EDTA$ (50 mL), and brine (50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on a silica gel column with hexane/EtOAc 1:1 as the solvent to give a white solid (400 mg, 67% yield). ^{1}H NMR (CD_3OD): 7.24 (dd, J = 3.6, 0.8 Hz, 2H), 7.14 (m, 5H), 7.08 (m, 4H), 6.97 (dd, J = 6.8, 2.0 Hz, 1H), 6.81 (m, 4H), 4.34 (s, 2H), 3.79 (s, 3H), 3.76 (s, 6H), 2.72 (t, J = 6.8 Hz, 2H), and 2.48 (t, J = 6.8 Hz, 2H) ppm. MS-ESI: $[M+H]^+$ (m/z, 536.4).

DMTr-S-acid (21)

To a solution of compound **20** (340 mg, 0.64 mmol) in 6 mL of DCM under N_2 were added 3-mercaptopropionic acid (56 µL, 0.64mmol) and TFA (67 µL, 0.83 mmol). The mixture was stirred at RT for 2 h. At that point, TLC indicated that compound **20** had disappeared. The volatiles in the reaction solution were removed under vacuum. The residue was purified by silica gel chromatography with DCM/MeOH (10:1) to give a colorless needle-like product (248 mg, 62% yield). ^{1}H

NMR ($CDCl_3$): 7.64 (d, J = 2.2 Hz, 1H), 7.27-7.11 (m, 10H), 6.78 (d, J = 8.8 Hz, 4H), 6.71 (d, J = 8.8 Hz, 1H), 6.50 (brs, 1H), 4.45 (d, J = 5.6 Hz, 2H), 3.77 (s, 6H), 3.72 (s, 3H), 2.77 (t, J = 6.8 Hz, 2H), 2.52 (t, J = 6.6 Hz, 2H), 2.40 (t, J = 6.8 Hz, 2H), 2.29 (t, J = 6.8 Hz, 2H) ppm. MS-ESI: $[M+H]^+$ (m/z, 624.0).

DMTr-S-ester (15)

Compound **21** (248 mg, 0.4 mmol) and *N*-hydroxysuccinimide (SuOH, 46 mg, 0.4 mmol) were dissolved in 8 mL of DCM. Then EDCI (77 mg, 0.4 mmol) was added at RT with stirring. After stirring at RT for 3 h, the reaction solution was diluted with DCM (60 mL), washed with water (5 mL) and brine (5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography with DCM/MeOH (20:1) to give white solid (204 mg, 72% yield). ^{1}H NMR ($CDCl_3$): δ 7.43 (d, J = 2.5 Hz, 1H), 7.26 (m, 5H), 7.22 (m, 5H), 6.82 (m, 4H), 6.46 (brs, 1H), 3.80 (s, 6H), 3.74 (s, 3H), 2.78 (m, 6H), 2.53 (m, 4H), 2.47 (m, 2H) ppm. MS-ESI: $[M+H]^+$ (m/z, 721.2).

2-Prop-2-ynyloxy-terephthalic acid dimethyl ester (5)

To a solution of compound **4** (24 g, 114 mmol) in 250 mL of CH_3CN was added K_2CO_3 (18.9 g, 137 mmol) and propargyl bromide (16.38 mL, 137 mmol). The reaction mixture was refluxed for 24 h under nitrogen. After cooling down to RT, reaction mixture was then poured into a mixture of EtOAc (100 mL) and 10 % HCl aqueous solution (10 mL). The organic phase was separated and washed with saturated $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. Removal of solvent to provide a wax-like light white solid (28.25 g, quantitative yield): ^{1}H NMR ($CDCl_3$) δ 7.84-7.82 (m, 1H), 7.71-7.69 (m, 1H), 7.782-7.779 (m, 1H), 4.85 (d, J = 2.0 Hz, 2 H), 3.95 (s, 3H), 3.92 (s, 3H), 2.56 (t, J =2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 166.0, 165.9, 156.6, 134.3, 131.6, 125.2, 122.3, 115.1, 77.6, 76.5, 56.9, 52.5, 52.4. MS (+ESI) m/z 249.1 $[M+H]^+$.

2-Prop-2-ynyloxy-terephthalic acid (6)

To a solution of compound **5** (3.0 g, 143 mmol) in 15 mL of CH_3OH was added 25 mL of sodium hydroxide solution (2M), the reaction mixture was refluxed for 3 h.

After removal of solvent by vacuum, the residue was acidified to pH 2 with 10% HCl solution. Solid was collected, washed with water, dried on vacuum to afford a white solid (2.11 g, 84%): ^{1}H NMR (DMSO-d_6) δ13.22 (brs, 2H), 7.71-7.73 (m, 2 H), 7.61-7.63 (m, 1H), 4.96 (d, J =2 Hz, 3H), 3.65 (s, 1H); ^{13}C NMR (DMSO-d_6) δ167.3, 167.0, 155.8, 134.7, 130.9, 126.9, 122.2, 114.7, 79.4, 79.2, 56.6. MS (-ESI) m/z 219.1 $[M-H]^-$.

N^1, N^4-Bis((9-(*N*-Boc-methylamino)methyl)anthracen-10-yl)methyl)-N^1, N^4-dimethyl-2-(prop-2-ynyloxy)terephthalamide (8)

To a solution of **7** (1.62 g, 4.4 mmol) and **6** (488 mg, 2.2 mmol) in 320 mL of dried DCM was added EDCI (820 mg, 9.0 mmol), HOBt (1.2 g, 8.9 mmol) and DMAP (108 mg, 0.9 mmol). After stirring overnight at room temperature under nitrogen atmosphere, the reaction mixture was washed with water and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel chromatography (DCM/CH_3OH 100/1) to afford a light yellow powder (1.76 g, 88%): ^{1}H NMR ($CDCl_3$) δ8.50 (s, 8 H), 7.58 (s, 8H), 7.28 (s, 1H), 7.12 (s, 1H), 7.02 (s, 1H), 5.86 (s, 4H), 5.55 (s, 4H), 4.55 (s, 2H), 2.54 (s, 3H), 2.51 (s, 6H), 2.32 (s, 3H), 1.57 (s, 18H); ^{13}C NMR ($CDCl_3$) δ170.3, 168.0, 153.2, 138.1, 130.9, 130.8, 130.1, 128.4, 128.1, 128.04, 126.2, 126.0, 125.9, 125.0, 124.5, 124.2,120.2, 111.4, 79.8,77.5, 76.1, 55.8, 53.3, 41.4, 38.5, 35.5, 33.9, 28.4. HRMS (+ESI): Calc. for $[C_{57}H_{61}N_4O_7]^+$ $[M+H]^+$ m/z 913.4540, Found 913.4566. MS (+ESI) m/z: 913.4 $[M+H]^+$.

N^1,N^4-Dimethyl-N^1,N^4-bis((9-((methylamino)methyl)anthracen-10-yl)methyl)-2-(prop-2-ynyloxy)terephthalamide (9)

A mixture of compound **8** (1.0 g, 1.1 mmol) and TFA (3 mL) in 12 mL of DCM was stirred at room temperature in the dark for 3 h. After removal of solvent, a mixed solvent of EtOAc/hexane 1:1 (20 mL) was added to the residue. Precipitate was generated and the solid was collected, washed with saturated $NaHCO_3$ solution and water, dried under vacuum to provide **9** as a light yellow solid (615 mg, 82%): ^{1}H NMR ($CDCl_3$) δ 8.43-8.39 (m, 8H), 7.57-7.52 (m, 8H), 7.29-7.26 (m, 1H), 7.09 (s, 1 H), 6.99-6.97 (m, 1H), 5.83-5.78 (m, 4H), 4.69 (s, 4H), 4.53 (s, 2H), 2.69 (s, 6H),

2.51 (s, 3H), 2.39 (s, 3H), 2.33 (s, 1H), 1.94 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 170.9, 168.6, 153.7, 138.6, 133.5, 131.6, 131.5, 130.5, 128.6, 128.0, 126.7, 126.5, 126.3, 125.5,125.4, 125.4, 125.0, 124.7, 120.7, 111.9, 78.1, 76.8, 56.4, 48.5, 42.4, 41.9, 37.6, 36.1, 34.5. HRMS (+ESI): Calc. for $[C_{47}H_{45}N_4O_3]^+$ $[M+H]^+$ m/z 713.3492, Found 713.3512. MS (-ESI) m/z 711.4 $[M-H]^-$.

Compound 11

To a mixture of compound **9** (105 mg, 0.14 mmol) and compound **10** (84 mg, 0.42 mmol) in 0.5 mL of DMSO was added DIPEA (0.12 mL, 0.7 mmol) and CuI (11 mg, 0.056 mmol). The reaction mixture was microwave-irradiated at 80 ^oC for 30 min under nitrogen atmosphere. To the reaction mixture, water was slowly added in (5 mL). Then the mixture was extracted with DCM. The combined DCM phase was washed with brine and dried over $MgSO_4$. Solvent was removed under vacuum and the residue was purified by silica gel chromatography (DCM/CH_3OH 10/3) to provide a light yellow solid (130 mg, 97%). ^{1}H NMR ($CDCl_3$) δ 8.44-8.43 (m, 8H), 7.58-7.53 (m, 9H), 7.08-6.96 (m, 3H), 5.87-5.69 (m, 4H), 5.10 (s, 2H), 4.76 (s, 4H), 4.20 (s, 2 H), 2.94 (s, 2H), 2.84 (s, 6H), 2.40 (s, 6H), 1.78 (s, 2H), 1.44 (s, 9H); MS (ESI) m/z 913.4 $[M+H]^+$

Compound 13

To a solution of compound **11** (200 mg, 0.11 mmol) in 12 mL of CH_3CN were added boronate **16** (124 mg, 0.44 mmol), K_2CO_3 (152 mg, 1.1 mmol), and NaI (4 mg, 0.022 mmol). The reaction mixture was stirred at room temperature for 16 h under nitrogen atmosphere in the dark. After filtering out the solid, the organic solvent was removed, and the residue was re-dissolved in DCM, washed with 5% $NaHCO_3$ solution and brine, and dried over $MgSO_4$. Solvent evaporation gave a crude product, which was re-crystallized with DCM/Et_2O to provide a light yellow solid (205 mg, 79% yield). ^{1}H NMR (DMSO-d_6) δ 6.80-9.15 (m, 28 H), 5.60-5.85 (m, 4 H), 5.15 (s, 2H), 4.53 (s, 4H), 4.25 (s, 2H), 3.95 (s, 4H), 3.31 (s, 6H), 2.46 (s, 6H), 2.31 (s, 2H), 1.34 (s, 9H). MS (ESI): m/z 1163.3 $[M-H_2O+H]^+$. HRMS (ESI): Calc. for $[C_{69}H_{73}B_2N_8O_8]^+$ $[M-H_2O+H]^+$ m/z 1163.5737, Found 1163.5760.

Compound 14

A mixture of compound **13** (240 mg, 0.2 mmol) and TFA (1.0 mL) in 10 mL of DCM was stirred for 4 h at room temperature under nitrogen atmosphere in the dark. After removal of solvent, the residual oil was dissolved in 3 mL of EtOAc. This was followed by the slow addition of 50 mL of Et_2O. The precipitate was collected and washed with saturated K_2CO_3 solution and water. Further purification by flash chromatography provided a white solid (133 mg, 73%): ^{1}H NMR (DMSO-d_6) δ6.80-9.40 (m, 30 H), 4.40-6.00 (m, 12 H), 3.40 (s, 6 H), 3.17-3.10 (m, 2 H), 2.82-2.74 (m, 2 H), 2.39 (s, 6 H). ^{13}C NMR (DMSO-d_6) δ: 158.9, 158.6, 136.2, 130.9, 130.6, 127.6, 127.0, 126. 8, 125.5, 125.0, 119.0, 116.0, 67.8, 51.3, 47.1, 36.8, 28.3, 21.9, 21.7. MS (ESI) m/z 1063.4 $[M-H_2O+H]^+$, 1081.5 $[M+H]^+$. HRMS (+ESI): Calc. for $[C_{64}H_{67}B_2N_8O_7]^+$ $[M+H]^+$ m/z 1081.5319, Found 1081.5363; Calc. for $[C_{64}H_{65}B_2N_8O_6]^+$ $[M-H_2O+H]^+$ m/z 1063.5213, Found 1063.5253.

Compound 16

In a 10-mL flask, compound **14** (60 mg, 0.05 mmol) and DMTr-S-ester (**15**) were dissolved in a mixture of 0.5 mL DMF and 1 mL DCM. Then Et_3N was added at RT in the dark. The mixture was stirred at RT overnight and then solvent was evaporated. To the resulting viscous residue was slowly added 10 mL of Et_2O to afford a suspension. The solid was filtered and washed with EtOAc to give crude product, which was purified by silica gel chromatography with DCM/MeOH (15:1) to give a white solid (30 mg, 36%). ^{1}H NMR (DMSO-d_6) 9.0 (m, 3H), 8.64-8.41 (m, 9H), 7.94-6.83 (m, 32H), 5.76-5.10 (m, 5H), 4.53-4.32 (m, 2H), 4.42 (s, 3H), 3.76 (s, 3H), 3.69 (s, 6H), 3.40 (s, 12H), 3.39-3.01 (m, 18H), 2.55-2.0 (m, 8H), 1.23 (m, 2H). MS-ESI: $[M-H-H_2O]^-$ (m/z, 1666.6) and $[M+H-H_2O]^+$ (m/z, 1669.2).

Tissue preparation and MALDI-IMS

Frozen tissue was sectioned and slides stored at -80 °C. For data collection, slides were removed from the freezer and rinsed in PBS for 5 min, followed by a rinse in water. The slides were then air-dried and overlaid with the boronolectin-trityl conjugate solution diluted in 100% methanol and incubated at room temperature for 2

h. Slides were then rinsed with 100% methanol to remove any unbound boronolectin-trityl conjugate, washed in PBS for 5 min, followed by a final water rinse. The slides were then allowed to air-dry and dessicated for 1 h before reading in the Ultraflex III MALDI-TOF (Bruker Daltonics), operated in reflectron mode with a raster width of 200 μm, to detect the trityl tags (m/z 500-600).

Immunostaining for Sialyl Lewis X

Immunostaining of frozen specimens was performed by the avidin-biotin peroxidase complex method using a Vectastain Elite ABC kit (Vector, Burlingame, CA). Frozen tissue was first treated with 0.3% hydrogen peroxide to block endogenous peroxide activity for 15 minutes. Sections were incubated in normal serum (provided in the kit) to block nonspecific binding and incubated with mouse monoclonal antibody to sialyl Lewis X, (clone KM93, Millipore, Billerica, MA) diluted 1:50 in 5% serum diluted in PBS for 1 h at room temperature. After washing in PSA, sections were then treated with biotinylated goat anti-mouse immunoglobulin G (IgG), followed by treatment with avidin-biotin-peroxidase complex, and stained with IMPACT DAB peroxidase substrate (Vector Labs) according to the supplier's protocol. Counterstaining was performed with Mayer's hematoxylin.

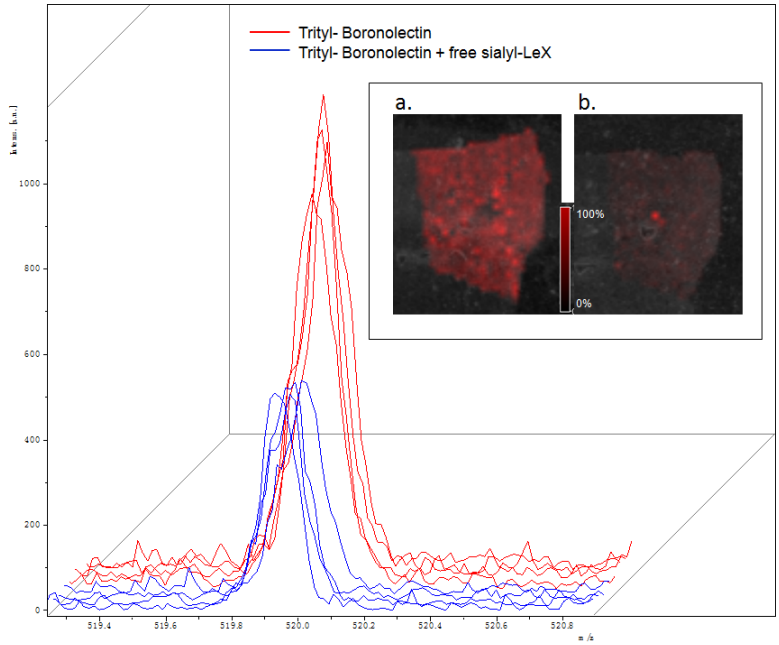
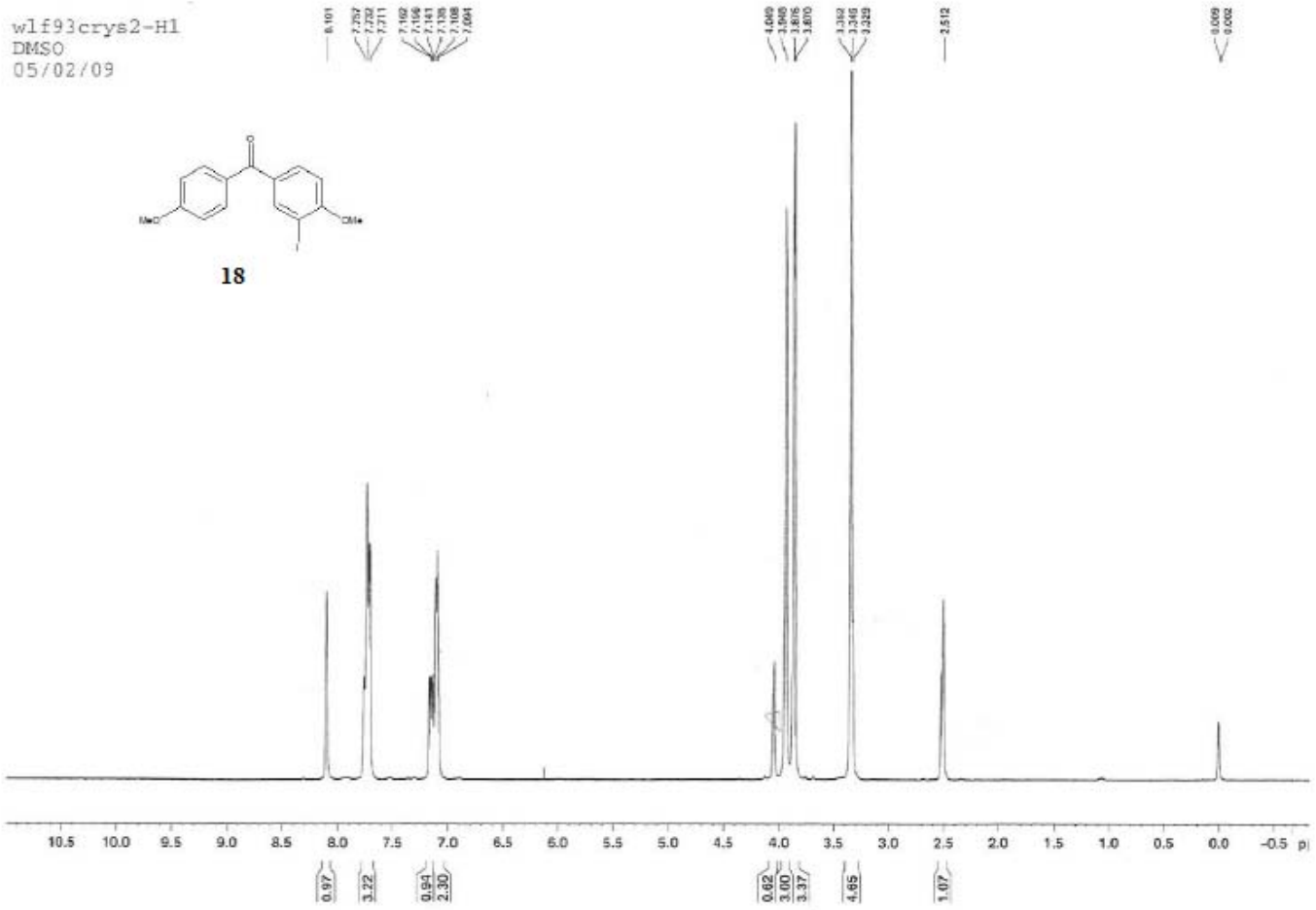


Figure S1. Borono-lectin trityl is blocked by carbohydrate.

Representative spectra exported from RCC regions after incubation overnight in the presence (blue) or absence (red) of 7 μM of sialyl Lewis X, followed by trityl-boronolectin-sialyl-LeX (1.5 uM) labeling and TIMS showing reduction in peak intensity with the carbohydrate was present. Inset) Images generated from RCC issue subjected to TIMS with the boronolectin-trityl alone (a) and with sialyl Lewis X (b), depicting the differences in signal intensity.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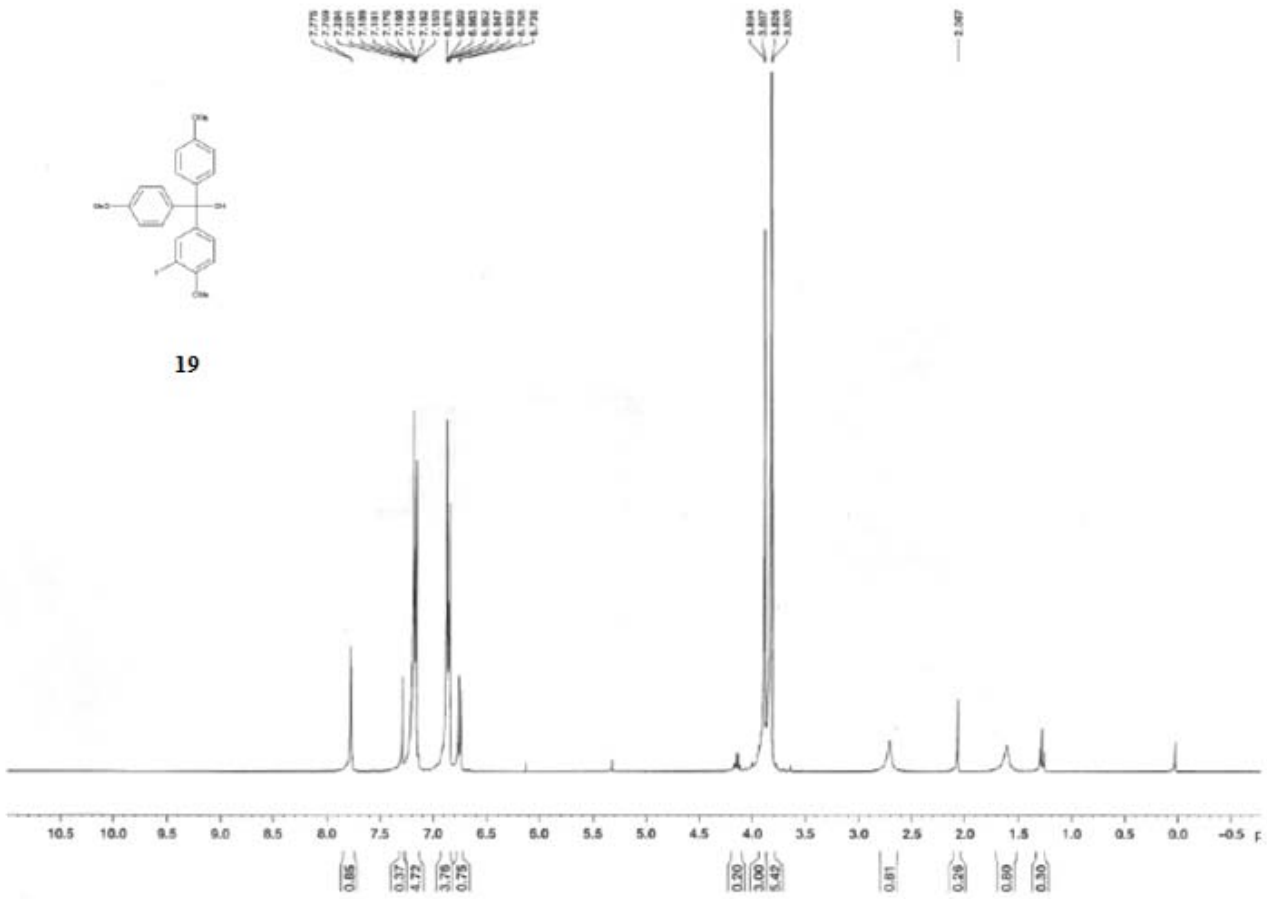

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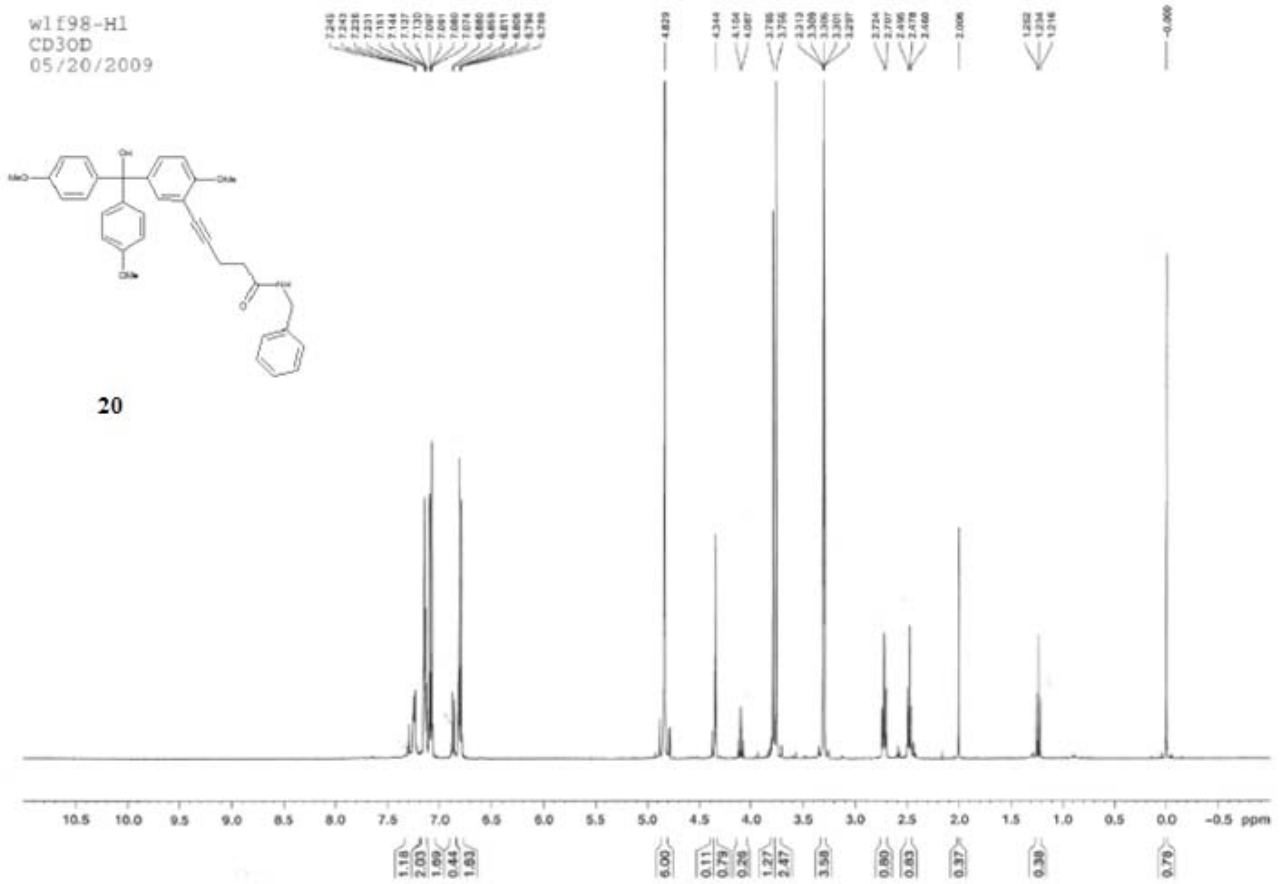
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CD3OD
05/20/2009
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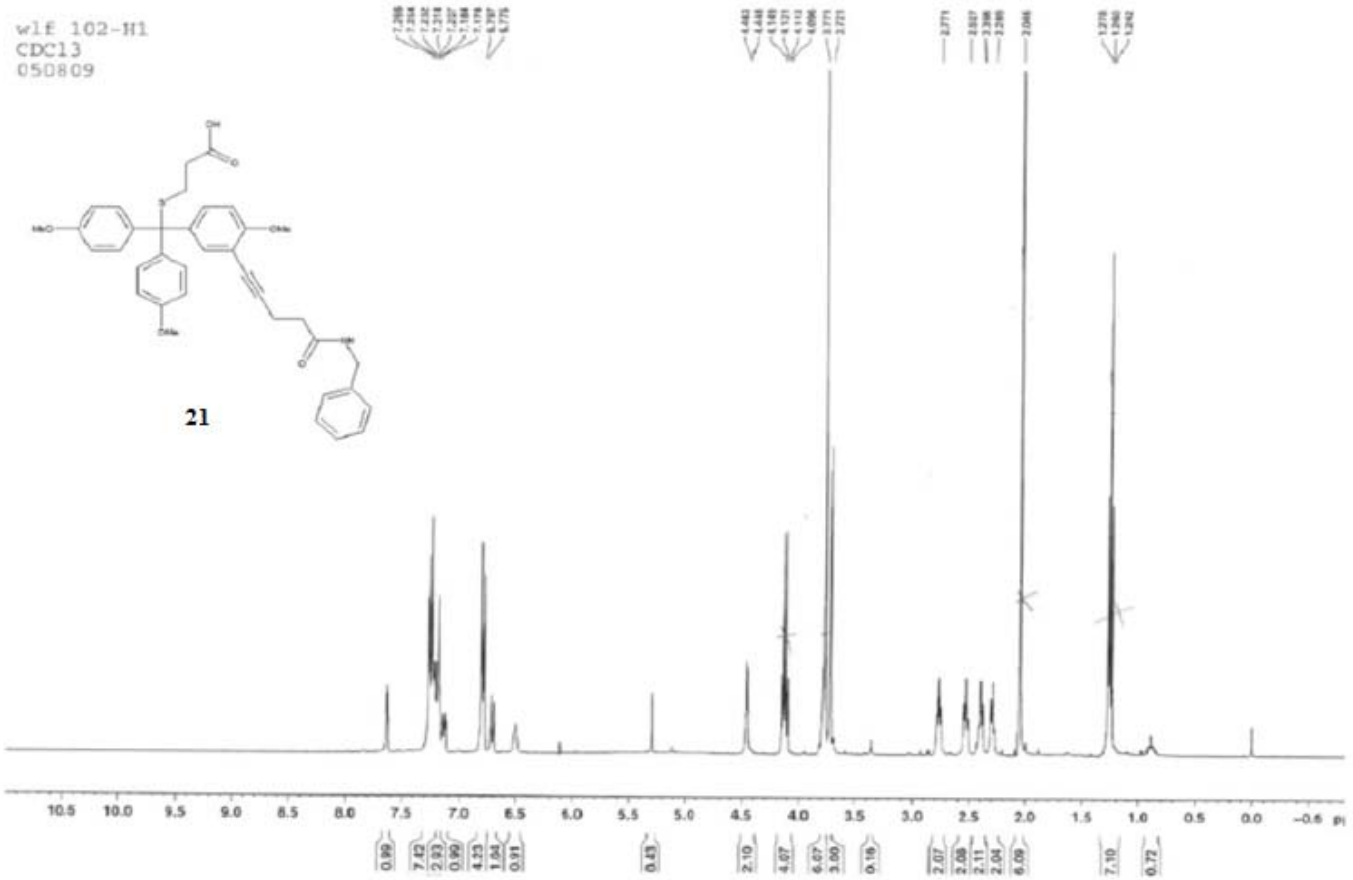

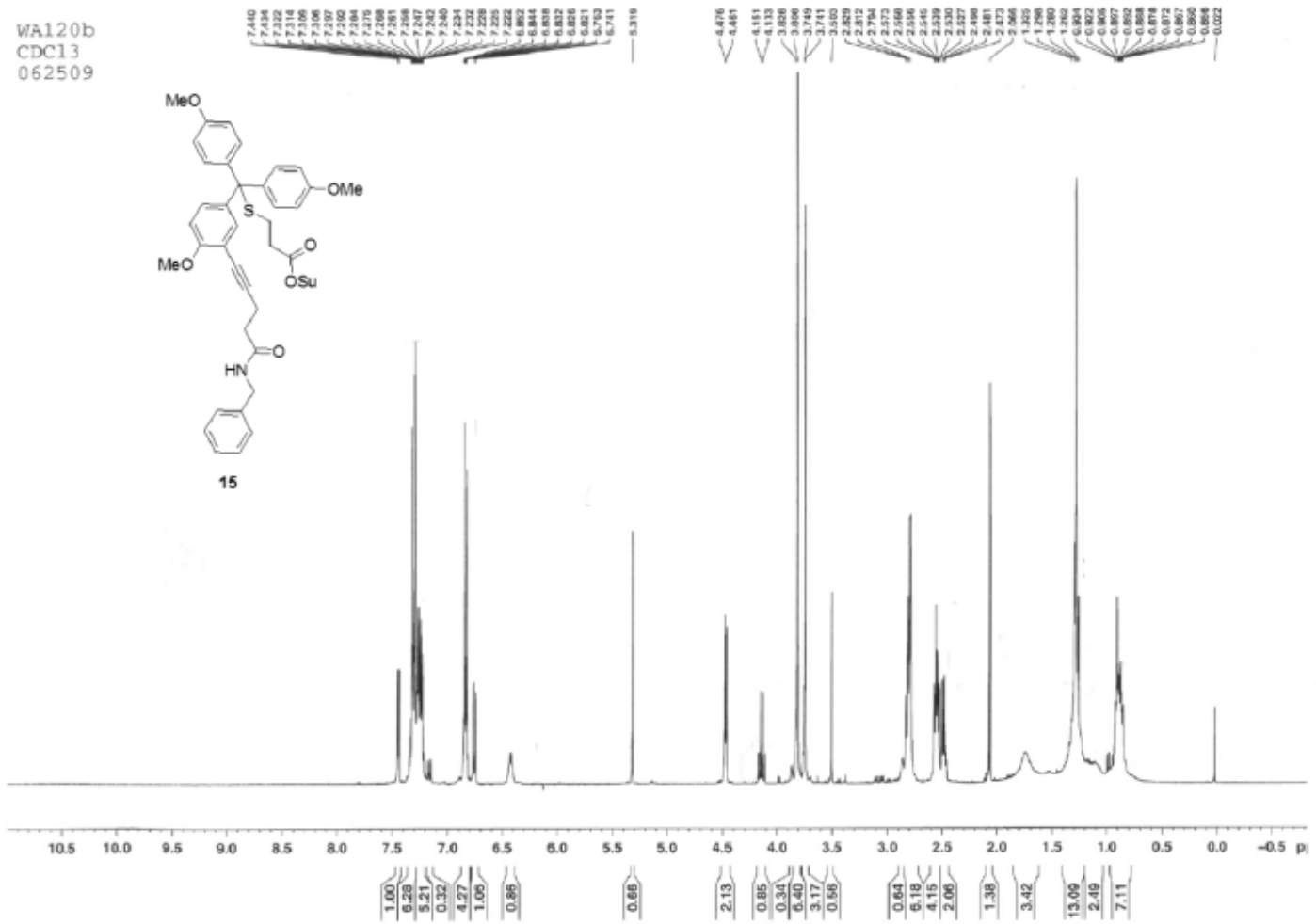
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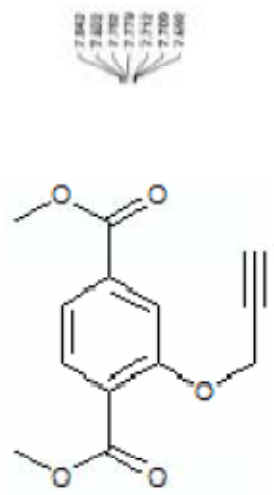
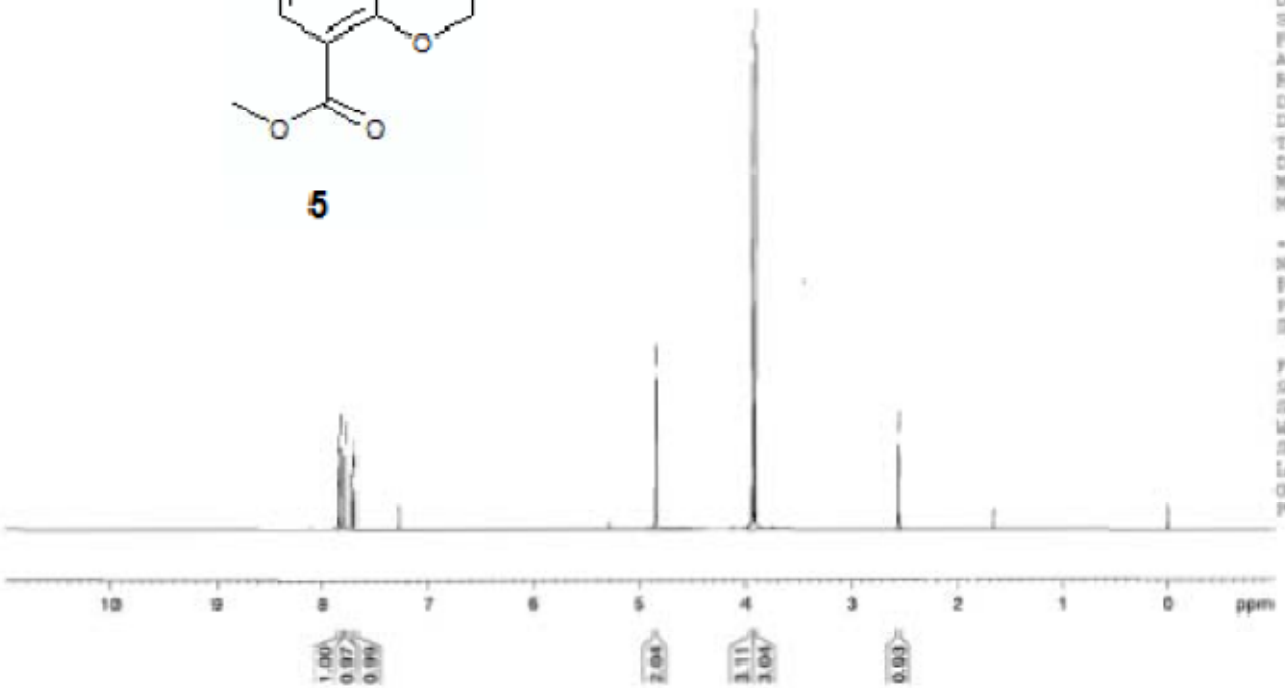
15

No title

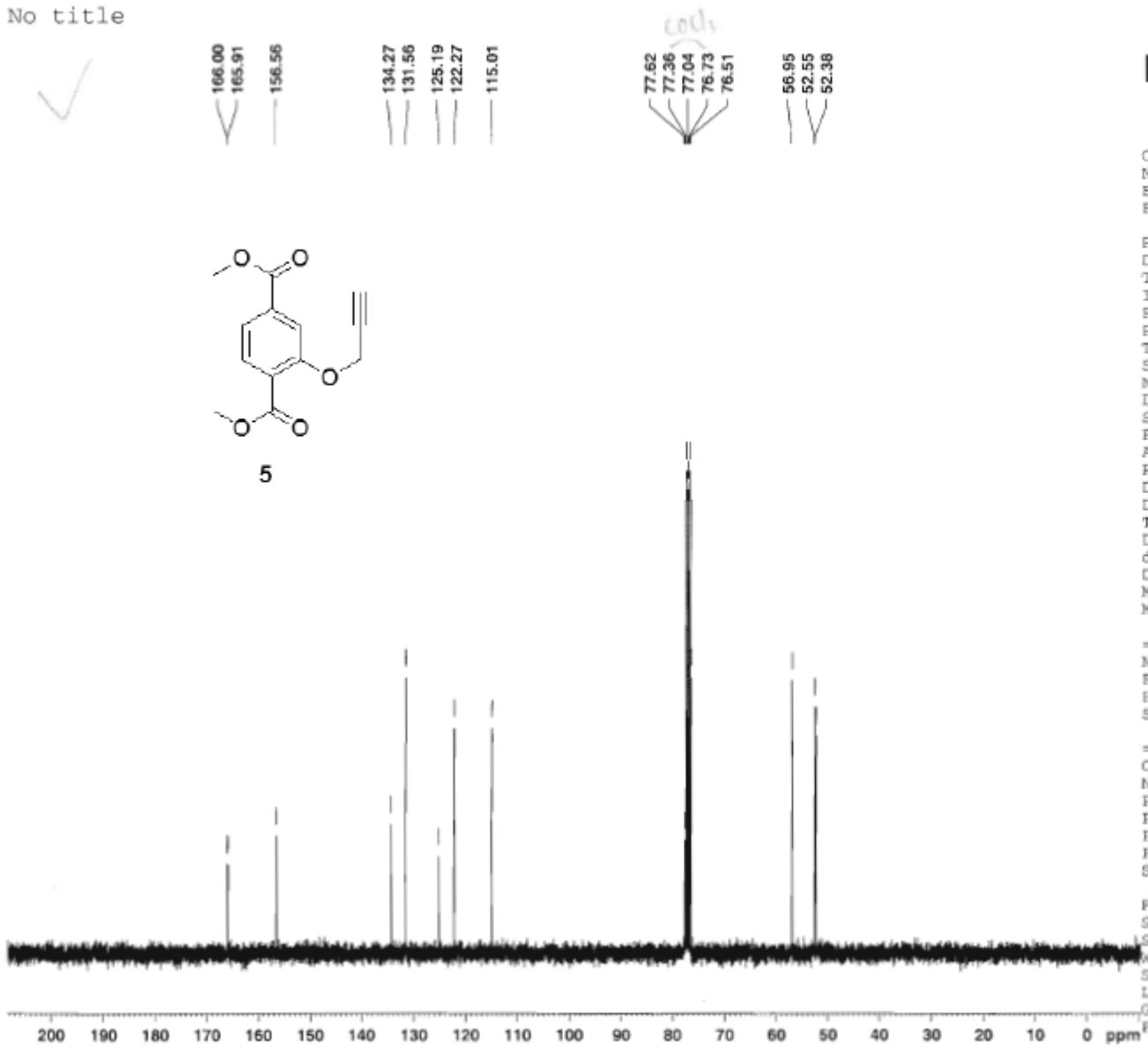


5



BRUKER

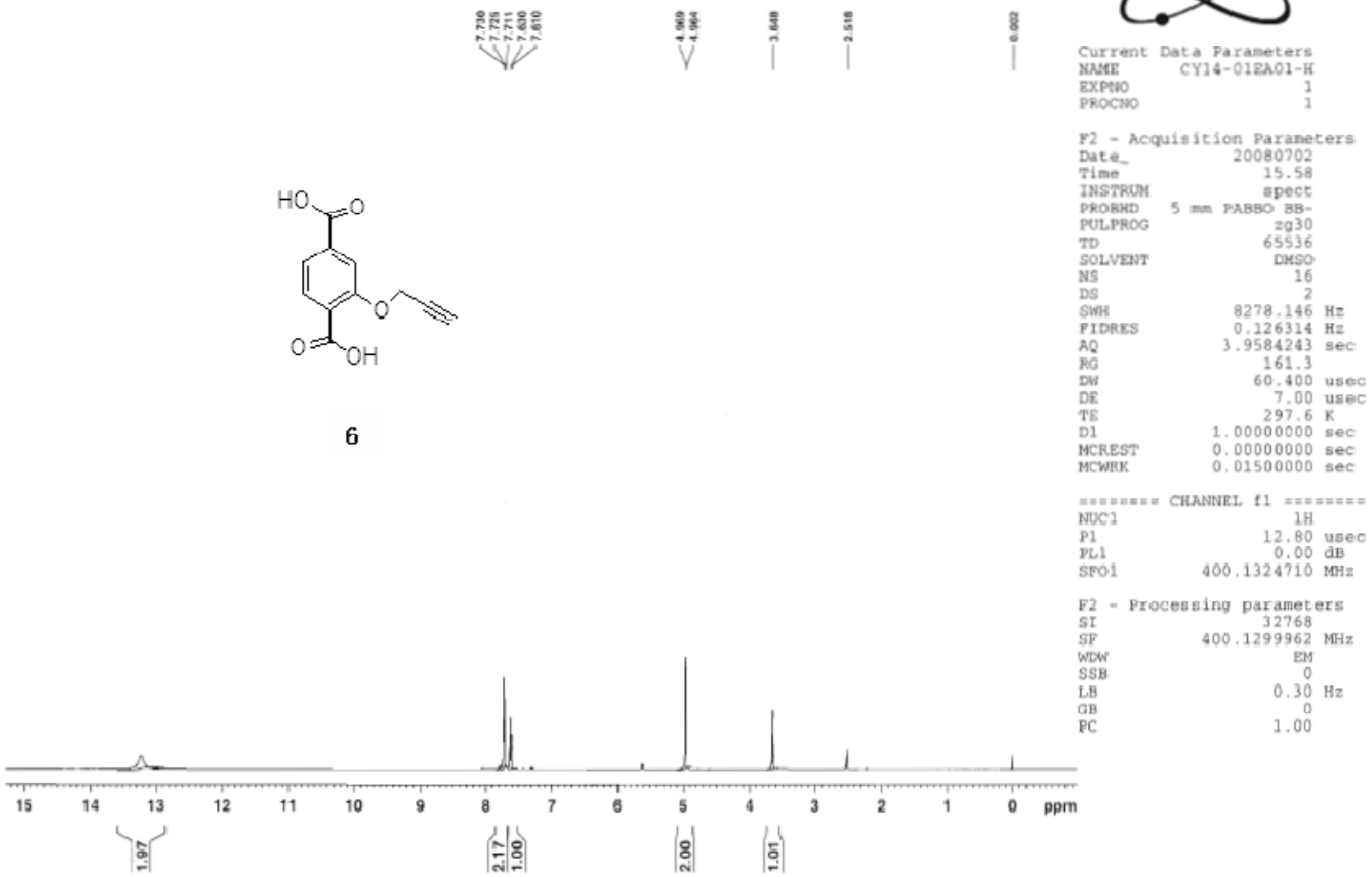
```
Current Data Parameters
NAME                  IV153
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20070221
Time                  17.55
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT                D2O
NS                        16
DS                         2
SWH               8278.146 Hz
FIDRES            0.126314 Hz
AQ                3.9584243 sec
RG                      181
DW                 60.400 usec
DE                  7.00 usec
TE                 298.5 K
D1              1.00000000 sec
MCREST          0.00000000 sec
MCWRK           0.01500000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                 12.80 usec
PL1                 0.00 dB
SFO1          400.1324710 MHz

F2 - Processing parameters
SI                     32768
SF            400.1300046 MHz
WDW                       EM
SSB                        0
LB                  0.30 Hz
GB                         0
PC                  1.40
```



BRUKER

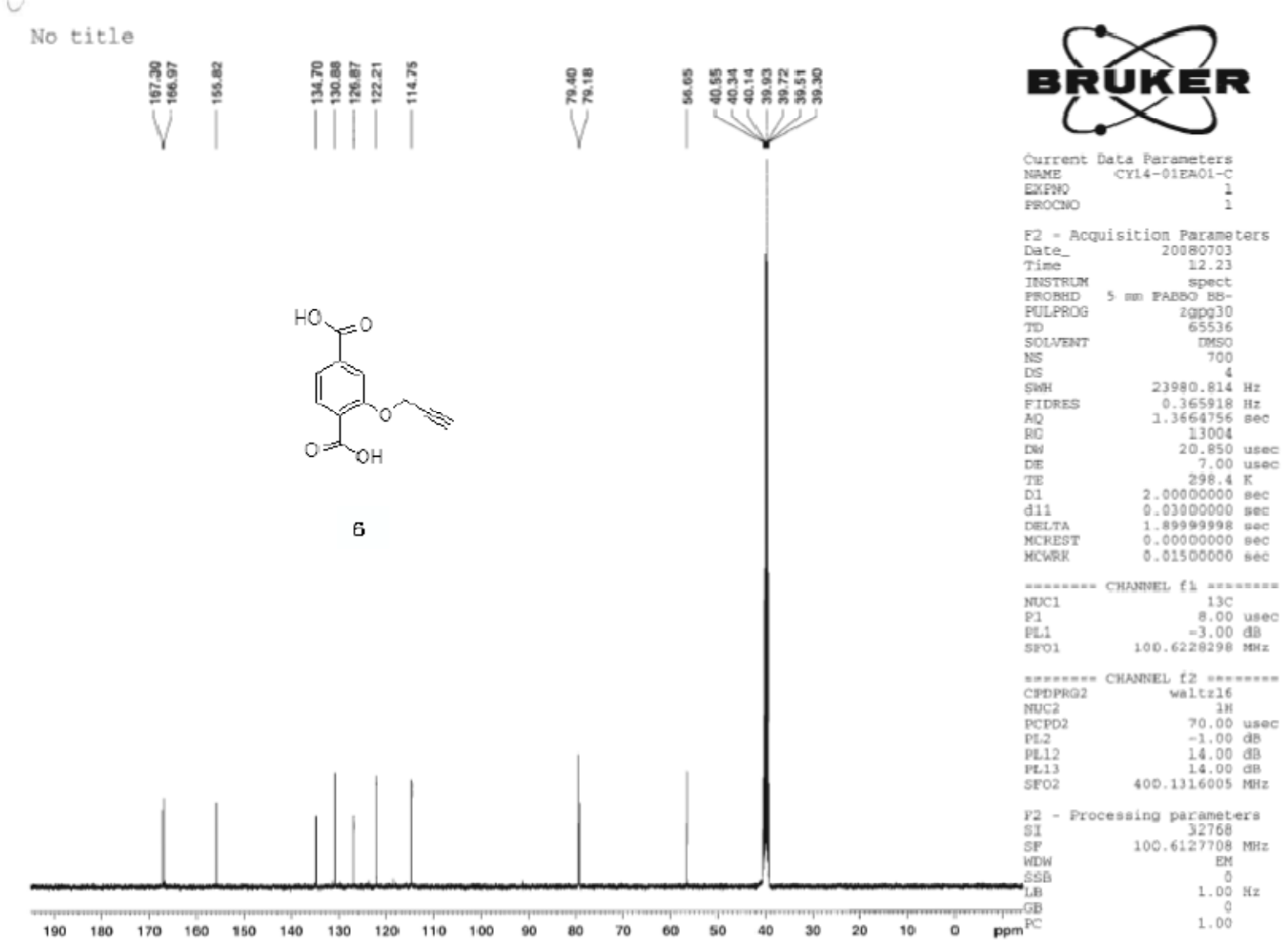
```
Current Data Parameters
NAME                  IV153
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20070221
Time                 17.57
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT              cdcl3
NS                      94
DS                       4
SWH              23980.814 Hz
FIDRES           0.365918 Hz
AQ               1.3664756 sec
RG                   32768
DW                  20.850 usec
DE                   7.00 usec
TE                  298.5 K
D1             2.00000000 sec
d11            0.03000000 sec
DELTA          1.89999998 sec
MCREST         0.00000000 sec
MCWRK          0.01500000 sec

======== CHANNEL f1 ========
NUC1                  13C
P1                   8.00 usec
PL1                 -3.00 dB
SFO1          100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
PCPD2               70.00 usec
PL2                 -1.00 dB
PL12                14.00 dB
PL13                14.00 dB
SFO2          400.1316005 MHz

F2 - Processing parameters
SI                   32768
SF            100.6127690 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.00
```

No title

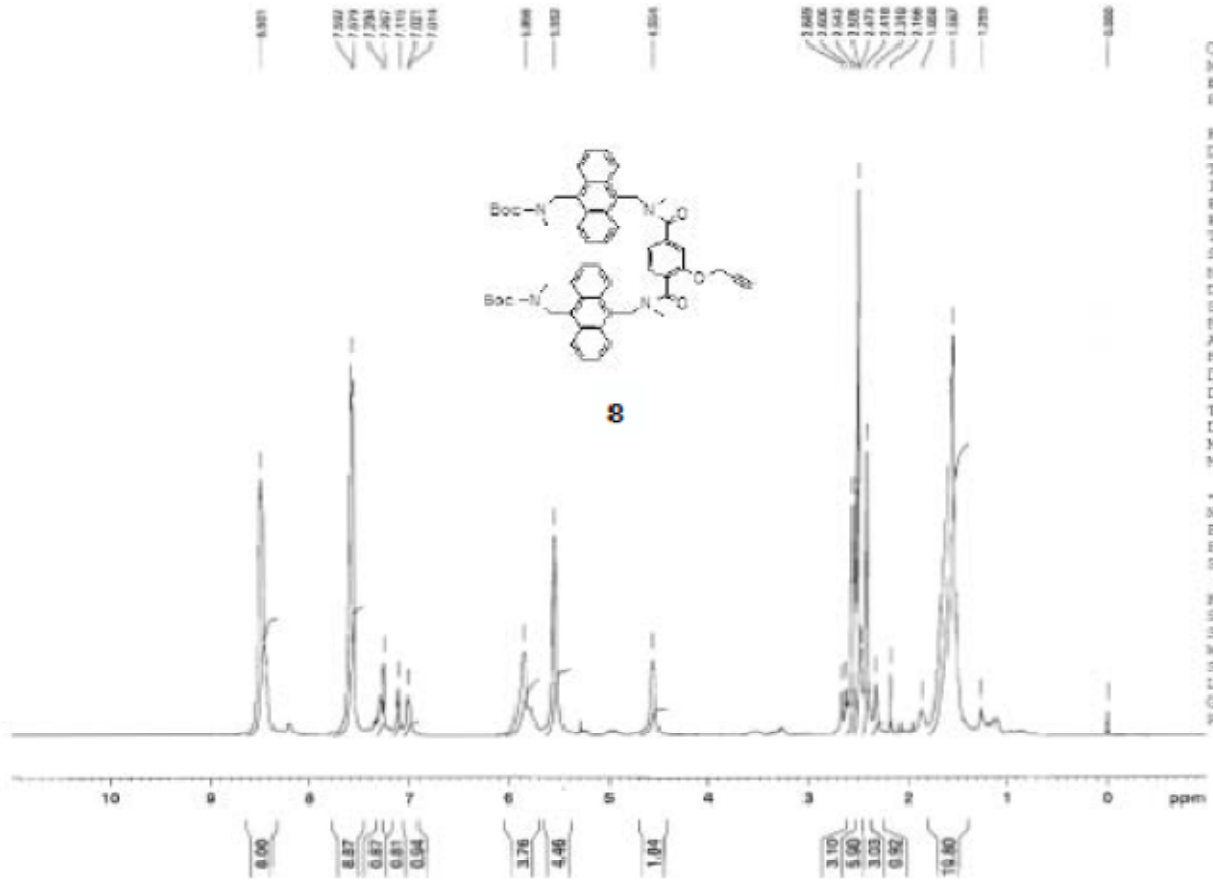


BRUKER

```
Current Data Parameters
NAME       CY14-01EA01-H
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20080702
Time               15.58
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9584243 sec
RG                 161.3
DW                60.400 usec
DE                 7.00 usec
TE                297.6 K
D1           1.00000000 sec
MCREST       0.00000000 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 12.80 usec
PL1                 0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF          400.1299962 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```


No title
167.30
166.97
155.82
134.70
130.88
126.87
122.21
114.75
79.40
79.18
56.65
40.55
40.34
40.14
39.93
39.72
39.51
39.30
HO
O
O
O
OH
6
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
BRUKER
Current Data Parameters
NAME CY14-01EA01-C
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20080703
Time 12.23
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 700
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 13004
DW 20.850 usec
DE 7.00 usec
TE 298.4 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
PL1 -3.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -1.00 dB
PL12 14.00 dB
PL13 14.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127708 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

No title

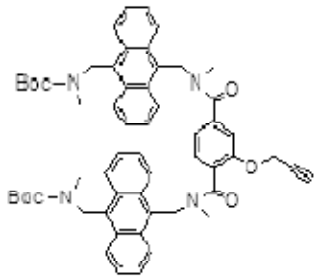


BRUKER

```
Current Data Parameters
NAME                  V15-10-15
EXPNO                         1
PROCNO                        1

F2 - Acquisition Parameters
Date_              20070322
Time                       9.21
INSTRUM                   spect
PROBHD    5 mm PABBO BB-
PULPROG                   zg30
TD                       65536
SOLVENT                  cdcl3
NS                          16
DS                           2
SWH                 8278.146 Hz
FIDRES              0.126314 Hz
AQ                  3.9584243 sec
RG                         64
DW                    60.400 usec
DE                     7.00 usec
TE                    297.8 K
D1                 1.00000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                      12.80 usec
PL1                      0.00 dB
SFO1              400.1324710 MHz

F2 - Processing parameters
SI                        32768
SF                400.1300066 MHz
WDW                          EM
SSB                           0
LB                       0.30 Hz
GB                            0
PC                       1.40
```

No title

170.29 168.34 168.01 155.70 153.16 138.07 130.90 130.85 130.14 128.41 128.13 128.04 126.24 126.02 125.93 124.96 124.50 124.21 120.53 120.17 111.39 79.78 77.66 77.47 77.32 77.00 76.68 76.15 56.35 55.82 53.34 45.66 43.26 42.49 41.86 41.35 38.48 35.55 33.92 31.73 30.16 28.42 13.00

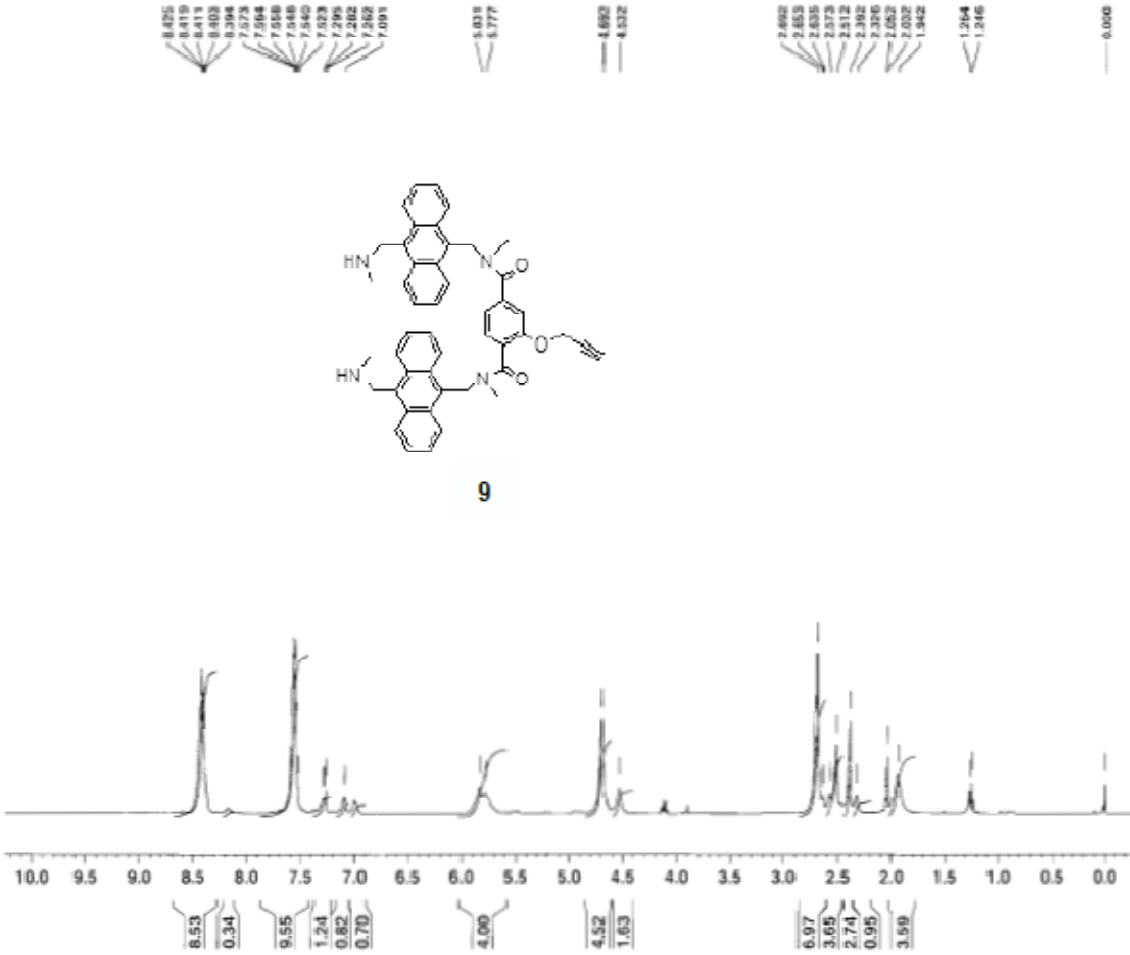


8

BRUKER

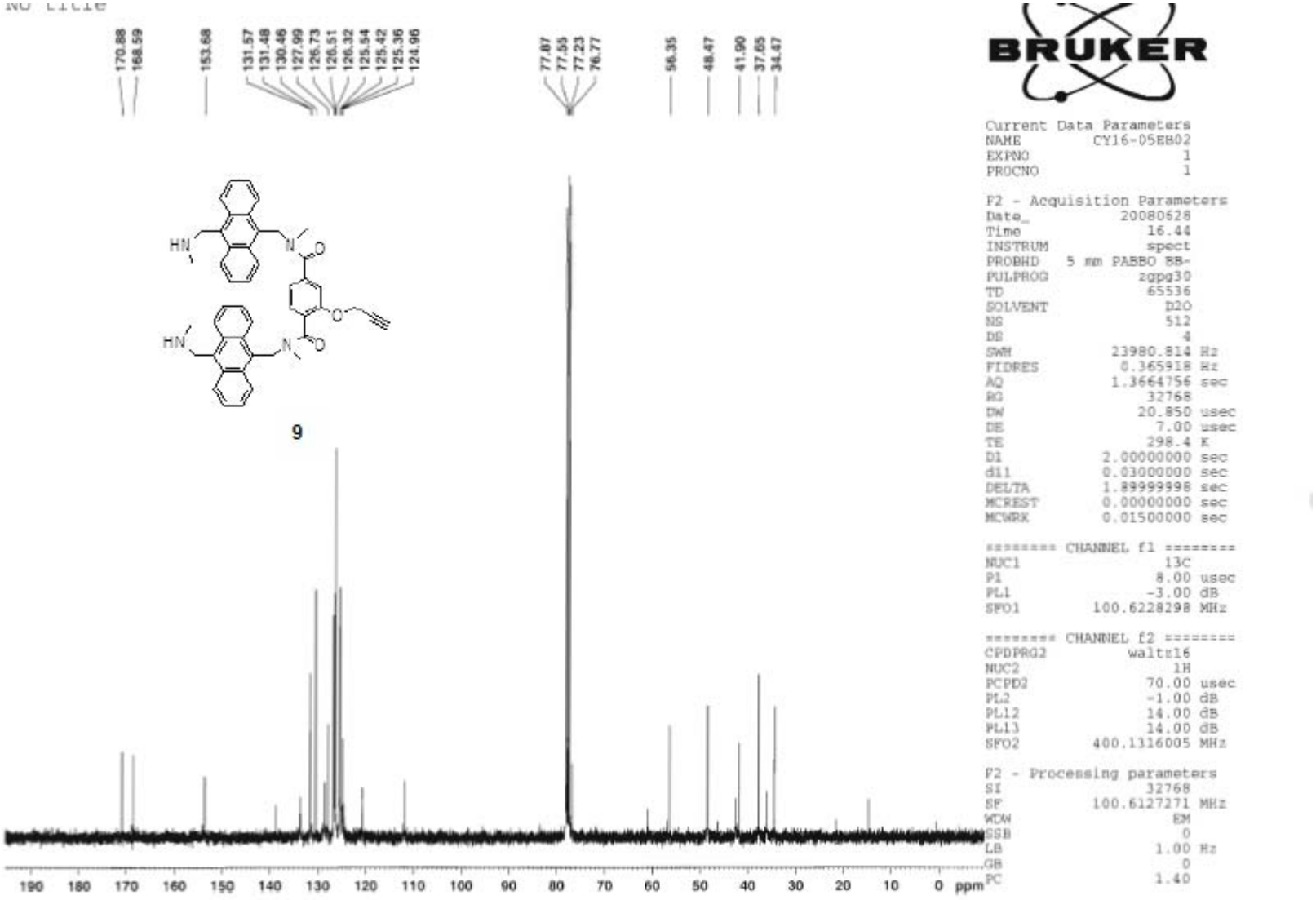
```
Current Data Parameters
NAME                  V6big-5-6
EXPNO                         2
PROCNO                        1

F2 - Acquisition Parameters
Date_              20070304
Time                  12.44
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT              cdcl3
NS                     436
DS                       4
SWH               23980.814 Hz
FIDRES             0.365918 Hz
AQ                1.3664756 sec
RG                   29193
DW                  20.850 usec
DE                   7.00 usec
TE                  297.8 K
D1               2.00000000 sec
d11              0.03000000 sec
DELTA            1.89999998 sec
MCREST           0.00000000 sec
MCWRK            0.01500000 sec

======== CHANNEL f1 ========
NUC1                    13C
P1                     8.00 usec
PL1                   -3.00 dB
SFO1          100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2                70.00 usec
PL2                  -1.00 dB
PL12                 14.00 dB
PL13                 14.00 dB
SFO2          400.1316005 MHz

F2 - Processing parameters
SI                     32768
SF            100.6127832 MHz
WDW                       EM
SSB                        0
LB                      1.00 Hz
GB                         0
PC                      1.00
```

No title



BRUKER

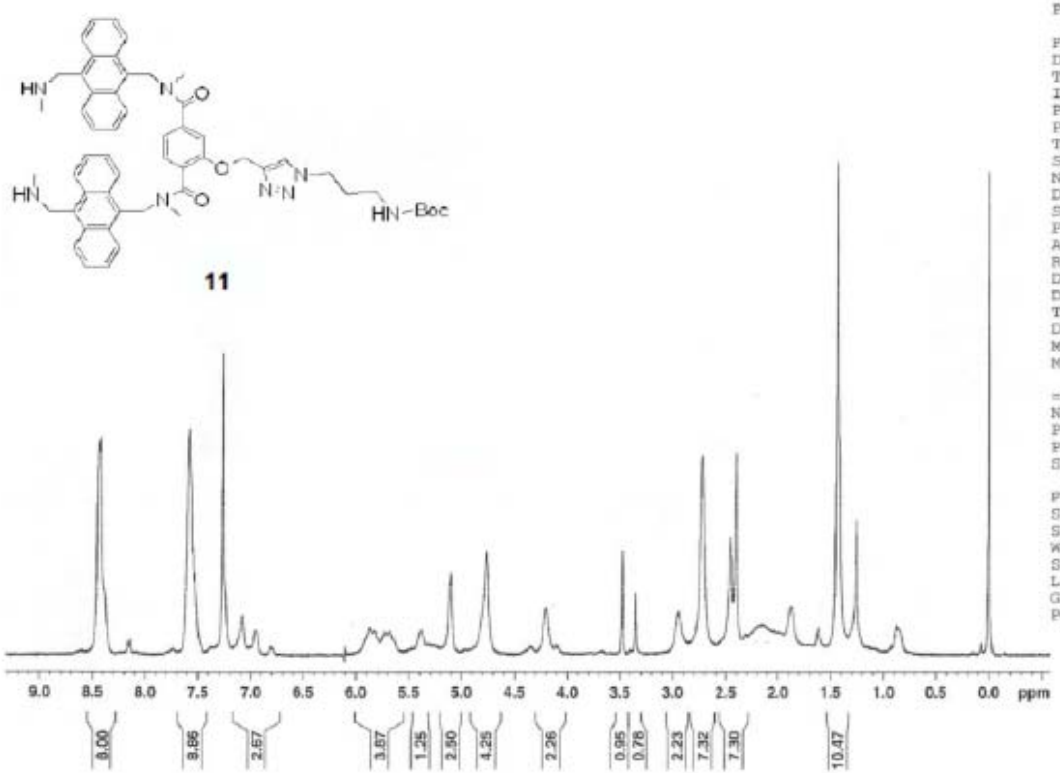
```
Current Data Parameters
NAME          CY16-05EB02
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20080628
Time                15.59
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9584243 sec
RG                     57
DW                  60.400 usec
DE                   7.00 usec
TE                  298.0 K
D1            1.00000000 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  12.80 usec
PL1                  0.00 dB
SFO1          400.1324710 MHz

F2 - Processing parameters
SI                  32768
SF            400.1300083 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```



BRUKER

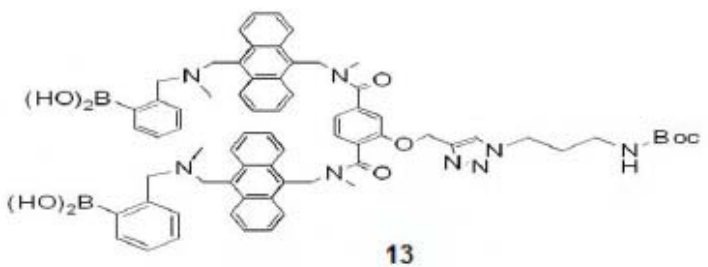
```
Current Data Parameters
NAME          CY16-05EB02
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20080528
Time               16.44
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG         zgpg30
TD                65536
SOLVENT             D2O
NS                  512
DS                    4
SWH           23980.814 Hz
FIDRES        0.365918 Hz
AQ            1.3664756 sec
RG                32768
DW               20.850 usec
DE                7.00 usec
TE               298.4 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                 8.00 usec
PL1               -3.00 dB
SFO1        100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                1H
PCPD2             70.00 usec
PL2               -1.00 dB
PL12              14.00 dB
PL13              14.00 dB
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                32768
SF          100.6127271 MHz
WDW                EM
SSB                 0
LB               1.00 Hz
GB                  0
PC                1.40
```

No title



BRUKER

```
Current Data Parameters
NAME        CY31-010A02-H
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20081009
Time                14.58
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9584243 sec
RG                   362
DW                60.400 usec
DE                 7.00 usec
TE                297.6 K
D1             1.00000000 sec
MCREST         0.00000000 sec
MCWRK          0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 12.80 usec
PL1                 0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300099 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



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BRUKER

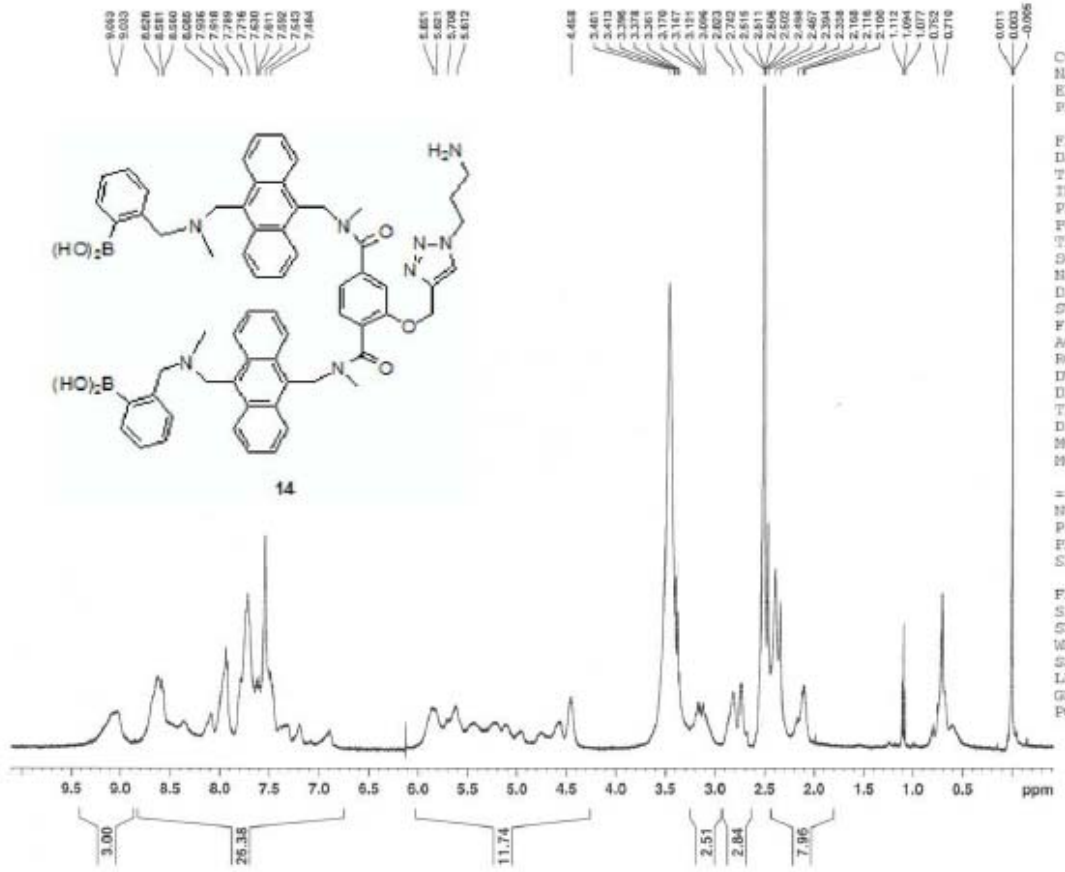
```
Current Data Parameters
NAME      CY32-01EA02-H2
EXPNO                  1
PROCNO                 1

P2 - Acquisition Parameters
Date_           20081016
Time               11.35
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           8278.146 Hz
FIDRES        0.126314 Hz
AQ            3.9584243 sec
RG                406.4
DW               60.400 usec
DE                7.00 usec
TE                299.8 K
D1           1.00000000 sec
MCREST       0.00000000 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                12.80 usec
PL1                0.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300008 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                1.00
```

No title



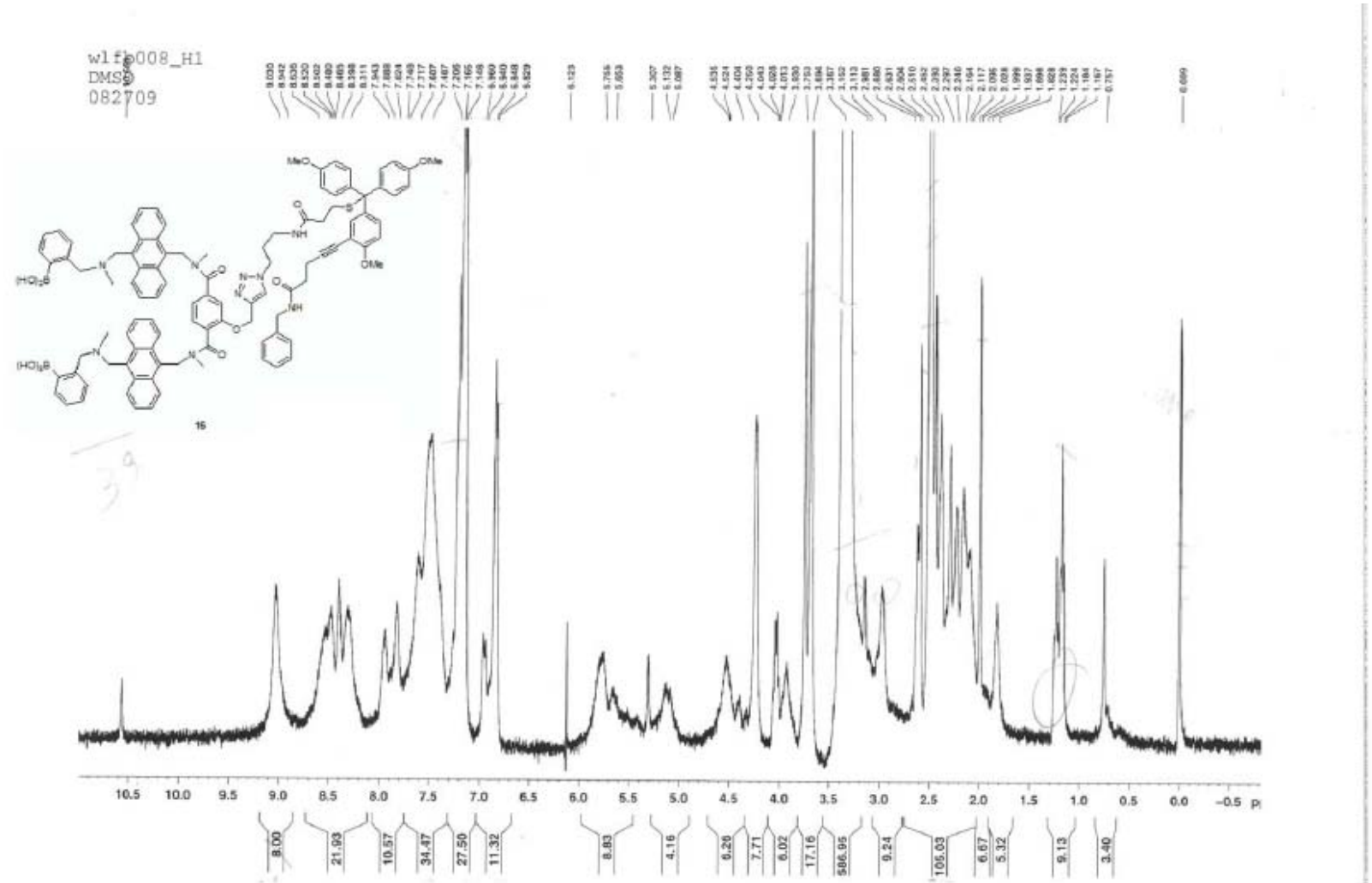
BRUKER

```
Current Data Parameters
NAME        CYJJ-01EA01-H
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20081016
Time                11.49
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9584243 sec
RG                   362
DW                60.400 usec
DE                 7.00 usec
TE                299.6 K
D1            1.00000000 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 12.80 usec
PL1                 0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300008 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```


wlf5008_H1
DMSO
082709
16
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
8.00
21.93
10.57
34.47
27.50
11.32
8.83
4.16
6.26
7.71
6.02
17.16
586.95
9.24
105.03
6.67
5.32
9.13
3.40

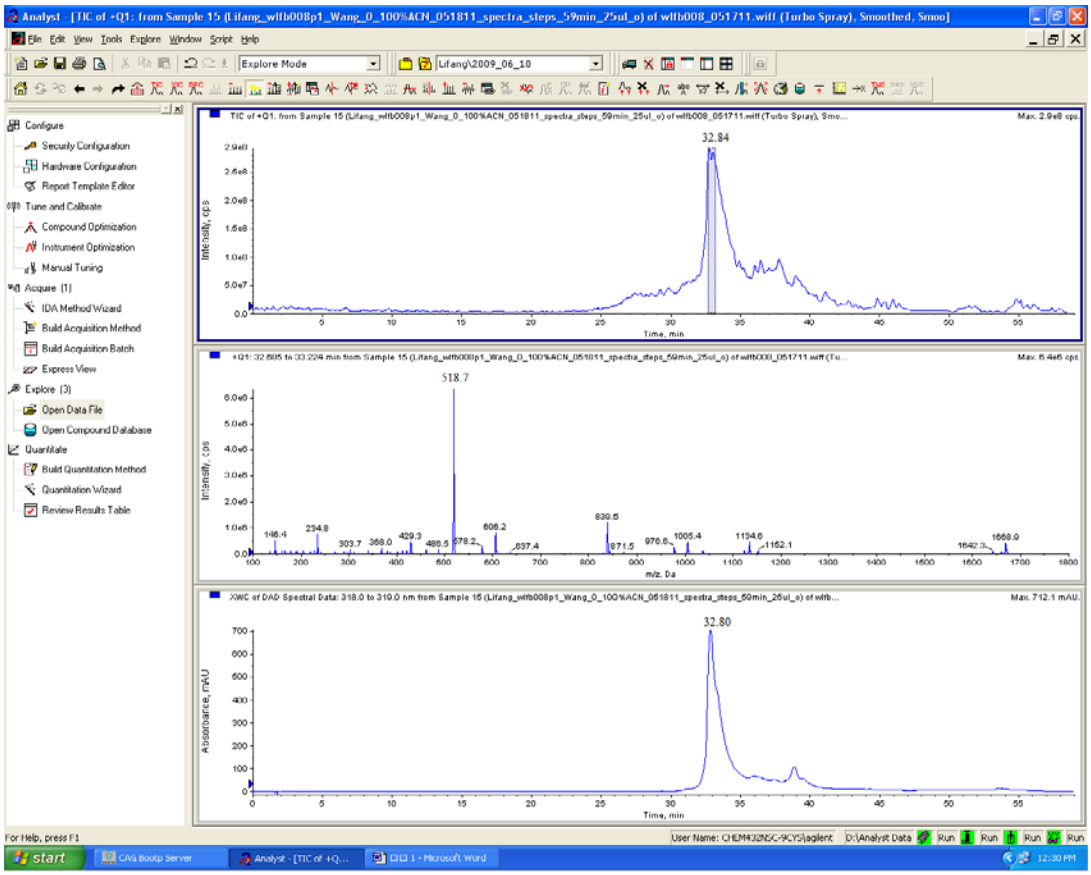


Figure S2 LCMS of compound **16**

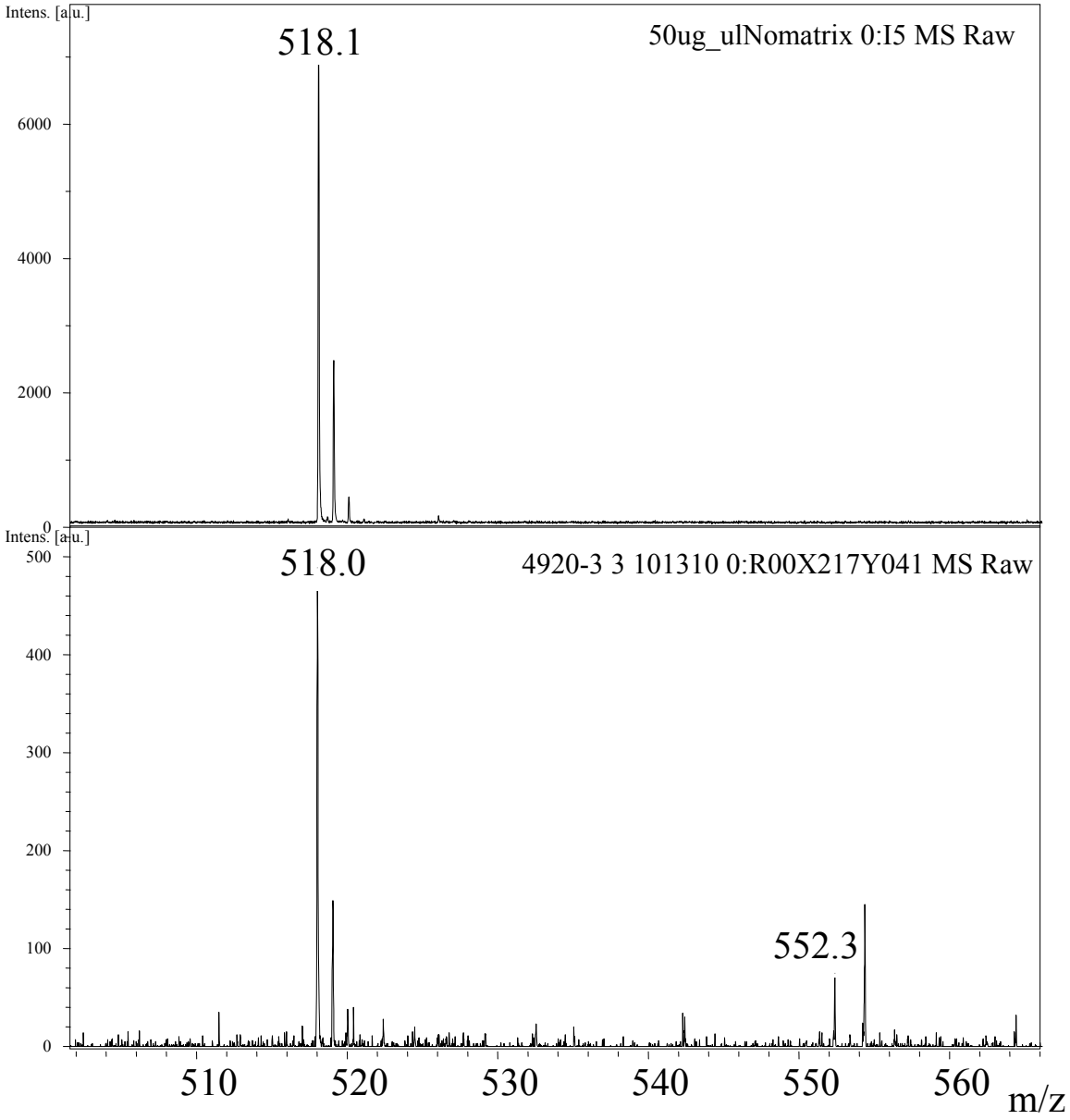


Figure S3 MALDI-IMS of compound **16** without matrix (top) and directly from tissue (bottom)